Molecular signatures of combination immunotherapy of prostate cancer using a Listeria-based PSA vaccine and radiation

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Background Radiation therapy (RT) has the potential to amplify immune responses triggered by tumor vaccines, including ADXS-PSA, a live-attenuated *Listeria monocytogenes* (*Lm*)-based vector expressing human PSA. Earlier observations suggest that the two treatment modalities cooperatively induce regression of syngeneic mouse prostate cancer cells expressing human PSA (TPSA23). However, the effects of different administration schedules and immune correlates of efficacy and tumor recurrence are poorly understood.

Methods We compared efficacy of different sequencing regimens of combination RT/vaccine treatments on TPSA23 tumor growth in syngeneic mice. Using the optimal sequencing protocol, tumors were collected to assess immune infiltrate and function during initial tumor regression (day 20) and, in a separate cohort, upon resumption of tumor growth (day 38). Correlates of treatment efficacy were determined by transcriptome analysis, phenotypic analyses of immune infiltrates and TCR sequencing.

Results We confirmed that combination RT/ADXS-PSA is superior to single modality treatments. Concurrent administration of RT with at least three vaccine doses was the most effective treatment schedule and was associated with enhanced T cell activation and robust IFNγ signatures in the tumor microenvironment. This was reflected in increased intratumoral CD4 and CD8 T cell infiltration in mice receiving the RT/vaccine combination. TCRβ chain sequencing revealed elevated and sustained T cell diversity in tumor tissues of RT/vaccine-treated mice, when compared with mice receiving single modality treatments. In these residual tumors resident and/or memory T cell phenotypic markers were increased. Transcriptome analysis of recurring tumors further revealed induction of PD-L1 as a function of treatment. Targeting of the PD-1/PD-L1 axis via a PD-1 blocking antibody administered in addition to radiation and ADXS-PSA (triple combination) further amplified tumor growth inhibition in mice receiving dual RT/vaccine therapy.

Conclusions Combining RT with the ADXS-PSA vaccine leads to effective tumor growth inhibition associated with robust, persistent antitumor immunity within the tumor environment. Transcriptome analysis during treatment revealed increased PD-L1 expression as a potential resistance mechanism and a PD-1 blocking antibody provided further therapeutic benefit. Collectively, these results support the rationale for combining *Listeria*-based vaccines with radiation in the clinic.

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